

a microarray approach. Identification of such genes and pathways will not only reshape the way we look at mesenchymal–epithelial interactions during development, but will also directly aid in the understanding of developmental causes of sensorineural hearing loss.

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Program/Abstract # 315

The Ldb1 transcriptional complex plays an essential role in regulating early mouse limb development

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The developing vertebrate limb is an excellent model for studying pattern formation and signal transduction. Many of the crucial genes that regulate growth and patterning of the limb are well defined. It is less clear however, how these molecules interact. Limbs develop from small buds consisting of a core of morphologically homogenous mesenchyme cells covered by a layer of ectoderm. Development of the limb bud depends on reciprocal interactions between the zone of polarizing activity and the apical ectodermal ridge. Sonic hedgehog (Shh) and fibroblast growth factors (FGFs) are key signaling molecules. LIM-homeobox (Lhx) genes play an important role in the transcriptional regulation of limb development. In a recent collaboration with the laboratory of Dr. Yingzi Yang, we were able to show that loss of Lhx2 and Lhx9 function resulted in patterning and growth defects of the developing limb. Similar but more severe phenotypes were observed when Ldb1, an obligatory cofactor of the Lhx genes, was abolished. In order to study the role of the Lhx/Ldb-complex in the regulation of signals during early limb development we compared the expression profile of mesenchymal cells in the hind limb buds of wild type and Ldb1 conditional knockdown mouse embryos at E10.5. Using microarray analysis followed by in situ hybridization, we were able to demonstrate that Shh and Fgf signaling pathways are downregulated at early stages of hind limb development. Biological processes affecting cell fate and cell death are upregulated when Ldb1 function is impaired. Thus, important signaling events in early limb development are controlled by Ldb1 and associated transcriptional regulators.

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Program/Abstract # 316

Chondroitin sulfate proteoglycans are required for proper Indian hedgehog signaling in the developing growth plate

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In contrast to the functional role of heparan sulfate proteoglycans (HSPGs), the importance of chondroitin sulfate proteoglycans (CSPGs) in modulating signaling pathways such as Hedgehog (HH), Wntless-type (Wnt) and fibroblast growth factor (FGF) remains unclear. To elucidate the importance of sulfated CSPGs in signaling paradigms required for endochondral bone formation, the brachy-morphic (bm) mouse was used as a model for undersulfated CSPGs. The bm mouse exhibits a postnatal chondrodysplasia caused by a mutation in the PAPS synthetase (*Papss2*) gene, leading to reduced levels of phosphoadenosine phosphosulfate (PAPS) and undersulfated proteoglycans. Biochemical analysis of the glycosaminoglycan (GAG) content in bm cartilage revealed preferential undersulfation of chondroitin sulfate (CS) chains and normal sulfation of heparan sulfate (HS) chains. Immunochemical analysis of the bm growth plate revealed abnormal Indian hedgehog (IHH) distribution marked by reduced IHH diffusion and abnormal aggregation. Consistent with

decreased IHH signaling, the bm growth plate had diminished *Ptch1* mRNA expression, reduction in the ratio of the *Gli1* activator to *Gli3* repressor mRNA expression, and decreased chondrocyte proliferation. IHH binding to defined GAG chains demonstrated that IHH interacts with CS, particularly chondroitin-4-sulfate. Furthermore, co-immunoprecipitation experiments showed that IHH binds to the major cartilage CSPG aggrecan via its CS chains. In sum, this study demonstrates an important function for CSPGs in modulating IHH signaling in the developing growth plate.

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Program/Abstract # 317

Early taste neuron maturation is dependent upon autocrine BDNF signaling

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Taste sensory neurons reside in the cranial ganglia of vertebrates. Ganglion neurons are generated from neural crest (NC) and neurogenic epibranchial placodes (EP), but taste neurons arise only from EPs (Harlow and Barlow, 2007). In mice null for BDNF, most taste neurons are lost by birth, and this loss is correlated with the absence of neurotrophic support from peripheral targets, the taste bud progenitors of the tongue (Nosrat et al., 1997). However, we find that BDNF is expressed in cranial ganglia (E9.5) before BDNF is expressed peripherally, and before taste neurites reach the lingual epithelium at E13. To test which ganglion cells produce BDNF early on, we crossed mice carrying a floxed BDNF lacZ reporter allele with either: 1) ectoderm-specific Cre mice (Crect, Yang et al., in prep) to assess EP neurons; or 2) Wnt1Cre mice to monitor NC cells. We find that BDNF is produced only by EP neurons, while NC cells (neurons and glia) lack BDNF. Further, only EP neurons express *trkB*, a BDNF receptor, suggesting that EP neurons require autocrine BDNF at early in ganglion formation. To test this, we created embryos null for BDNF in EP neurons only, and made several measures of initial gangliogenesis. Total neuron number and ganglion size were unaffected in EP *bdnf* null mice at E11.5, in contrast to loss of neurons postnatally. Instead, EP neuron size was reduced in mutants, suggesting an effect on neuron maturation. Consistent with this idea, initial neuronal differentiation appeared unaffected in mutants, but later maturation was impaired.

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Program/Abstract # 318

A second role for the receptor CLV1 in stem cell repression in developing fruit of *Arabidopsis*

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In crops such as tomatoes and peppers, the largest fruit also have the greatest number of fruit organs. Studies in tomato show that increases in both cell division and organ number are responsible for larger fruit, but the molecular mechanisms through which these components integrate and contribute to overall patterning and size is still not clear. Fruit arise from floral meristems (FM) derived from inflorescence meristems (IM), stem cell-containing structures, and studies in *Arabidopsis* indicate that when extra cells are present in FM, extra fruit organs are produced. Mutation of individual components

of the stem cell-repressing CLAVATA signaling pathway results in an increase of cell number in both IM and FM. Through the characterization of new alleles in the receptor-kinase *CLAVATA1* (*CLV1*), we identified a second role for *CLV1* in the regulation of fruit organ number via the repression of stem cell proliferation during later stages of fruit development. This later role is temporally distinct from the previously known role of *CLV1* in IM and FM, and our analysis of *CLV1* expression indicates that *CLV1* is not limited to the meristem. We show that loss of *clv1* activity in developing fruit leads to an accumulation of stem cells as shown by the ectopic activation of the stem cell-promoting factor *SHOOTMERISTEMLESS* in developing fruit, and that extra organs are produced from these stem cell containing regions. Our results indicate that in addition to its role in the FM, *CLV1* is also acting to repress stem cell proliferation in developing fruit.

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Program/Abstract # 319

Analysis of signaling downstream of ephrin-B1 during development

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Ephrin-B1 controls craniofacial, skeletal, and neural developmental processes by multiple signaling mechanisms. In addition to Eph receptor tyrosine kinase engagement and activation of downstream signaling, ephrin-B1 can act as a receptor and signal into the cell in which it is expressed. We have taken an approach integrating genetic analysis, proteomic, and transcription profiling methods to identify the downstream signaling pathways responsible for mediating ephrin-B1 function during development. By generating mice harboring point mutations that specifically ablate reverse signaling while maintaining forward signaling, we find that reverse signaling by a PDZ-dependent mechanism is critical for axon guidance of the corpus callosum. Reverse signaling is dispensable for skeletal and craniofacial development, however, indicating that forward signaling controls these processes. We have therefore sought to identify downstream components of this forward signaling network during development by two methods. First, utilizing mass-spectrometry, we have identified multiple downstream phosphorylation targets of ephrin-B1 forward signaling. Second, microarray analysis provides a profile of downstream transcriptional targets of ephrin-B1 signaling. Integrating these datasets provides insight into the molecular mechanisms by which ephrin-B1 controls distinct developmental processes.

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Program/Abstract # 320

Schnurri functions in an evolutionarily conserved mechanism for BMP target gene regulation

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BMPs regulate gene networks in a wide variety of different biological contexts. In frog (*Xenopus*) and zebrafish embryos BMP signaling functions in ventral specification of the mesoderm and ectoderm. BMP signaling leads to activation of specific target genes in a cell type dependent manner via partnering of Smad proteins with different transcription factors. Different Smad-interacting transcriptional part-

ners direct the Smads to different gene promoters in depending on the cell type. Working in *Xenopus* gastrulae, we previously identified BMP-responsive cis-regulatory elements (BREs) in the promoter proximal regions of the direct BMP target genes *vent2* and *id3*. These BREs were shown to function in *Drosophila* embryos, suggesting an evolutionary conservation in DNA recognition mechanisms. Examination of the *Xenopus* BRE sequences revealed binding sites with a geometry shown to bind the fly Smads (Mad and Medea), together with Schnurri, a large zinc finger transcription factor. In *Xenopus*, we found that these BREs bind the vertebrate Smads and Shn1, one of the three vertebrate orthologs of fly Schnurri, and suggest that Schnurris play roles in BMP signaling in vertebrate cells. We have created both a zebrafish line carrying a BRE-driven GFP reporter and BRE-lacZ mice. We are using these to reveal endogenous patterns of Schnurri activity. We have also made Shn1-specific antibodies and have performed chromatin immunoprecipitation experiments to examine the in vivo genome-wide binding of Shn1 in the frog gastrula.

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Program/Abstract # 321

Analysis of Capza3 localization reveals temporally discrete events during the acrosome reaction

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The starting point of development is the fusion between sperm and egg. It is well established that sperm fuse with the egg through the equatorial/postacrosomal region. On top of this observation and the requirement of two proteins (CD9 in the egg and Izumo in the sperm) very little is known about this fundamental process. Sperm proteins that are important for sperm maturation and function localize to various sites in this highly compartmentalized cell. Some of these critical proteins change their localization during sperm processes. We have previously shown that Izumo changes localization during the acrosome reaction in an actin dependent manner. This finding is significant because Izumo's localization in acrosome-intact sperm is not compatible with the known location of the initiation of the fusion between the sperm and the egg. To further understand the actin-mediated changes in protein localization during the acrosome reaction, the distribution of the sperm-specific plus-end actin capping protein Capza3 was analyzed. Like Izumo, Capza3 shows a dynamic pattern of localization; however, these movements follow a different temporal pattern than the changes observed in Izumo. In addition, the actin polymerization inhibitor Latrunculin A was unable to alter Capza3 movement. These results suggest that movements of Capza3 are independent of actin polymerization. Due to the specific changes in Capza3 localization observed during the acrosome reaction, this protein could be used as an indicator of early and late acrosome reaction events and thus aid in the dissection of this complex process.

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Program/Abstract # 322

Importance of the ERK/MAPK pathway in syncytiotrophoblasts differentiation

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The mammalian genome contains two ERK/MAP kinase genes, *Map2k1* and *Map2k2*, encoding dual-specificity kinases